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PRINCIPAL INVESTIGATOR: Massimo Loda, M.D.

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute
Boston MA 02115

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Massimo Loda, M.D.

E-Mail: massimo_loda@dfci.harvard.edu

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Table of Contents

Introduction

Currently, primary human tumor material is insufficient due to small size, multifocality and difficulty of visualization at macroscopic examination. This essentially prevents extensive studies aimed at distinguishing indolent and aggressive organ-confined prostate cancers. Understanding the molecular alterations governing tumorigenesis and cancer progression is the first step necessary for the design of effective and targeted therapies. For this reason, in recent years considerable efforts have been devoted to generate clinically relevant models of prostate tumors. As a result, a number of cell lines and in vivo models have been developed. To date, however, there is no model in which all aspects of human PCa progression can be mimicked.

It is important to note that the overwhelming majority of organ-confined human prostate cancers display a luminal phenotype, characterized by expression of androgen receptor (AR), PSA and luminal-type keratins. The established human cell lines most commonly utilized in prostate cancer research (LNCaP, DU145, PC-3, LAPC-4 and LAPC-9) are derived from metastases and only few of them are androgen-sensitive [1, 2]. In the last 20 years a variety of methodologies have been attempted for the purpose of establishing primary epithelial cell cultures displaying a luminal phenotype reflective of the human primary prostate tumor of origin, but attempts have been largely unsuccessful. The possibility exists that small populations of contaminating non-malignant cells (invariably present in the human tissue sample) can populate and ultimately replace transformed cells in vitro. When transformed cells are indeed obtained from prostate primary adenocarcinomas they rarely form tumors in nude mice. Finally, primary cells from prostate cancers are mostly diploid and do not show relevant chromosomal alterations [3].

In recent years many mouse models of prostate cancer have been generated by injecting established cell lines (mostly LNCaP and PC-3 sublines) subcutaneously or orthotopically [4-7], while a few mouse xenografts have been established with human benign and malignant prostate tissues in both orthotopic and subrenal capsule sites [8, 9]. Experimental evidence suggests that orthotopically transplanted tumors may be the most appropriate system because of the potential to reproduce the microenvironment and metastatic patterns of human prostate cancer [10]. In addition, the relationships between nerves, stroma and epithelium in prostate cancer seem to be relevant as interactive processes requiring signaling systems and regulatory mechanisms that play major roles in the development, the local progression and subsequently the metastatic diffusion of prostate tumors [11-13]

We proposed to graft directly primary localized human prostate tumors samples orthotopically in immunodeficient mice and also to use these tumors for purifying to homogeneity in vitro epithelial cells showing a malignant behavior as assessed by growth in soft agar. This system, capable of mimicking the morphology, micro-environment, growth patterns and dissemination of human tumors will provide a new preclinical model to optimize therapeutic protocols and drug validation.

Body

Specific Aim 1. To collect fresh human prostate tissue samples and isolate primary prostate epithelial cell cultures for generating xenografts in nude mice (months 1-18).

To accomplish the first task, we set the best technical conditions to manipulate, implant and image fresh human prostate tissues or cells in immunodeficient mice (nude) in the orthotopic site by using as a positive control LNCaP cells, a common metastatic prostate cancer cell line, and subcutaneous xenografts derived from these cells (Fig. 1). Currently, we are also setting up an *in vivo* imaging system to monitorize prostate tumors growing in mice grafted with these prostate cancer cells infected with a retroviral luciferase construct.

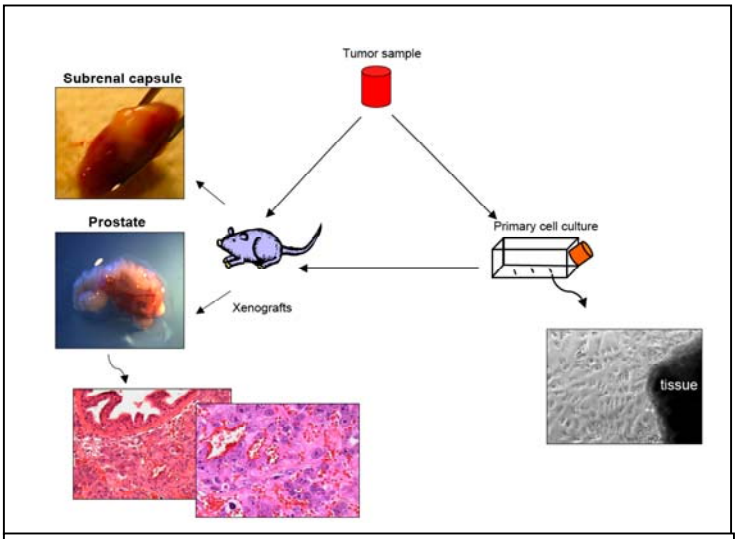


Fig.1- Example of prostate tumor generated by orthotopic injection of LNCaP cells and transplanted in the subrenal capsule of a nude mouse.

We have thus far collected 61 fresh human prostate samples from patients undergoing radical prostatectomy (Brigham and Women's Hospital) for clinically localized prostate cancer (exempt IRB 05-036). Due to the small size of most tumors and difficulty to grossly identify prostate cancer, only 37 of our samples showed 40-90% of tumor at histopathological exam (frozen section) and were utilized as follows:

a) **Five tumors** were implanted in the orthotopic site of nude mice (both normal and tumor tissues respectively in the two anterior prostate lobes) after isolation of epithelial cells. Tissues were mechanically disrupted and shortly digested with collagenase at 1mg/ml concentration in Hank's balanced salt solution. The cell pellet was resuspended in 20 mcl of serum deprived-medium (Iscove's Modified Dulbecco's medium, IMDM), mixed with 20 mcl of matrigel and immediately injected in the immunodeficient host. These 5 samples were used to graft 19 mice. A testosterone pellet (12.5 mg, 90 day-release) was implanted subcutaneously in all mice.

b) Eight primary cell cultures (4 from benign prostatic hyperplasia (BPH) and 4 from cancer) were isolated from multiple samples of **4 radical prostatectomy surgical specimens**, two of which belonged to the group of tumors described in a).
To isolate these cells we used the same method described in our preliminary data.
Two mm^3 of tumor and benign tissues were cultured in standard T25 flasks with 2 ml of Iscove's modified Dulbecco's Medium (IMDM) enriched with 20% Fetal Bovine Serum (FBS) for at least two weeks [14]. These conditions favored epithelial cell growth. At 80% confluence these primary cells were passaged and characterized for expression of epithelial markers (cytokeratins) and androgen receptor. Cells that showed a luminal epithelial phenotype (negativity for p63, positivity for cytokeratin 8 and androgen receptor) were plated in soft agar. Importantly, growth in soft agar was specific of tumor-derived cells.
Three isolated primary cell cultures were injected in the anterior prostate of 15 nude mice (5 mice/each). To this purpose $1x10^6$ cells were mixed with matrigel as decribed in a). A testosterone pellet (12.5 mg, 90 day-release) was implanted subcutaneously in all mice.

All these mice **(n=34)** were sacrified 1 and 3 months after implantation and prostate anterior lobes microdissected for histopathologic and immunohistochemical analyses.
The injection of epithelial cells obtained either by enzymatic digestion following mechanical disruption of the tissue or by *in vitro* passage resulted in the presence of small foci of human prostate malignant cells in the context of mouse stroma, mostly around peripheral nerves and ganglia (Fig.2). No macroscopically evident tumors were found.

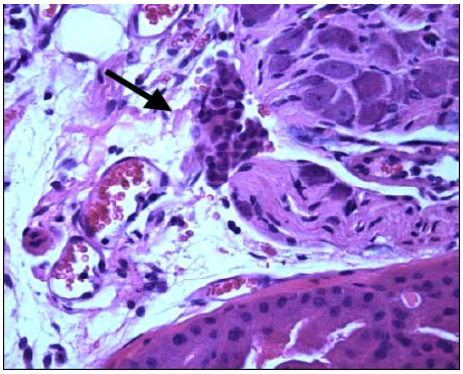


Fig.2- Example of human prostate tumor cells growing around a mouse nerve.

c) Because of these results, we recently implanted **30 human prostate tumors** in both orthotopic and subrenal capsule sites (Fig. 1) of **75 immunodeficient mice**, to compare the efficiency of growth. Moreover, we used both nude and NOD/SCID mice, that show different immunodeficiency profile. Two mm^3 of fresh tumor or normal tissue was implanted in mouse within a few hours of surgery. No enzymatic digestion of the tissue was performed.

Thus far, 30 mice implanted under the renal capsule with 10 human tumors (3 mice per tumor) were sacrified no sooner than 3 months after implantation. Three out of ten tumors grew (totally 9 mice), showing a phenotype consistent with the original implanted human tissues (high expression of both androgen receptor and PSA were maintained) (Fig.3). Importantly, these human tumors showed high pathological grade in the original frozen section. All mice bearing human tissues were monitored through 3 months after implantation, by monthly serum PSA measurement (ELISA). Interestingly, the mice (n=3) grafted with one of these 3 human tumors presented the highest levels of PSA (11-13 ng/ml) versus an average of 2-4

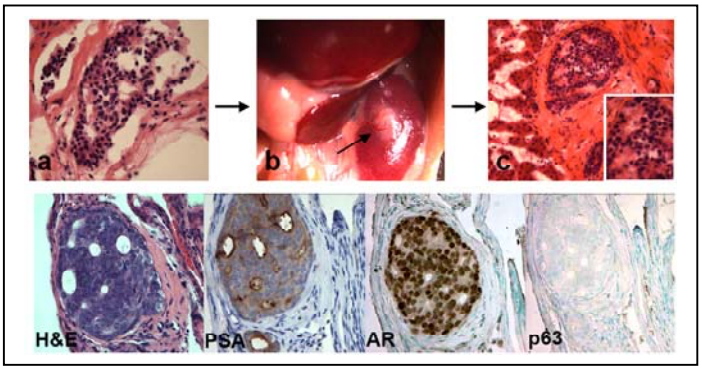
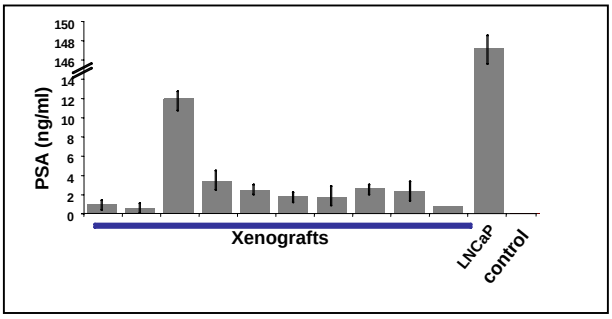


Fig.3- Human prostate tumor (a) grafted in the subrenal capsule site (b, c) of a nude mouse. Histological exam (H&E) and immunostaining for PSA, AR and p63 were performed on paraffin sections of the mouse xenograft.

ng/ml in the others (Fig.4). No difference in terms of growth was found between nude and NOD/SCID mice. Unfortunately, a few mice showed presence of calcifications and epithelial degeneration, in the subrenal xenograft, suggesting that other factors related to the tissue manipulation or to the immunodeficient mouse strain need to be redesigned.

Fig.4- Serum PSA levels in nude mice implanted with 10 human prostate tumors (3 mice per tumor), LNCaP cells (positive control) and negative control (wild-type mouse).



Based on these results, we plan to implant fresh tissues in different strains of immunodeficient mice, i.e. SCID beige, that are NK cells-deficient, or in irradiated (400 rads in unique dose) nude mice.

Specific aim 2. To perform gene expression profiling and SNP analysis on original prostate specimens and derived primary prostate cells before and after implantations in nude mice (months 6-34)**.**

Tissues have been microdissected by Laser Capture Microdissection (LCM) and RNA and DNA extracted and stored.

Specific Aim 3. To generate mouse orthotopic prostate cancer xenografts from engineered human primary prostate cell cultures (months 18-34)**.**

We will accomplish this task in the next year.

Key research accomplishments

1) collection of 61 human prostate tissue samples
2) implantation of 37 human prostate tumor samples in 109 immunodeficient mice
3) isolation of 8 primary epithelial cell cultures, 4 from BPH and 4 from prostate cancer

Reportable outcomes

None

Conclusion

Work in progress. High grade human prostate tumors grow in nude or SCID mice, others need additional factors. We will implant fresh tissues in different strains of immunodeficient mice, i.e. SCID beige, that are NK cells-deficient, or in irradiated (400 rads in unique dose) nude mice. Moreover, we will co-inject prostate epithelial cells with human prostate fibroblasts, to recreate the original microenvironment.
The development and characterization of such experimental model provide an invaluable resource to conduct new studies on the biology of primary localized prostate cancers and on the influence of the genetic background on drug resistance and sensitivity.

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